Bull. Environ. Contam. Toxicol. (1990) 44:636–642

Bulletin of Environmental Contamination and Toxicology

Effect of Chemically Contaminated Marine Sediment on Naupliar Production of the Marine Harpacticoid Copepod, *Tigriopus californicus*

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There is a growing body of evidence indicating that chemically contaminated sediments in urban bays and estuaries pose a significant threat to the productivity of these important marine habitats (Swartz et al. 1982; Malins et al. 1984; Myers et al.1987; Johnson et al. 1988). Particularly at risk are benthic species which live in direct contact with the sediment. However, nondemersal species are also at risk via the food chain and by direct contact with resuspended sediment particulates.

There are substantial data on the lethal and sublethal effects of aqueous contaminants on a variety of aquatic species. In contrast, there is very limited information on the toxic effects of the generally water-insoluble sediment-associated contaminants. The data that are available deal mainly with acute effects and there exists a critical need for information on chronic lethal and sublethal consequences of exposure to contaminated sediments.

In the present communication we report a series of experiments in which the harpacticoid copepod, *Tigriopus californicus,* was exposed to sediments from urban and nonurban bays, and reproductive success was evaluated. This species was selected for study as it is widely distributed along the West Coast of North America (Dethier 1980), and as a group, copepods are an important component of the marine food chain. In addition, the relatively short reproductive life span of this species (Huizinga 1970; Burton 1985) makes it particularly amenable for studies of reproductive success. Antia et al. (1985) observed impaired naupliar production of *T.californicus* after adults were exposed to the insecticide diflubenzuron. Here, we report reduced and irregular naupliar production as a consequence of exposure to chemically contaminated sediments from urban waterways.

MATERIALS AND METHODS

A stock culture of *T. californicus* was obtained from the National Marine Fisheries Service Laboratory at La Jolla, California. Copepods were maintained and tested on a 16L:8D cycle at 18°C in 3 μm filtered seawater with a salinity of 26-28‰. Copepods were fed a suspension of the alga *Isochrysis galbana.* Stock cultures were allowed to acclimate for 3 mon before testing began. Tests were conducted with females of the same age (cohort) bearing their first egg clutches. Cohort preparation was begun by placing 50 ovigerous females from the stock culture in a 1 L beaker with seawater. After 2 d females were removed, leaving behind a group

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of newly hatched nauplii of similar age (± 24 hr). This cohort was reared to sexual maturity and after 21 d females carrying their first egg clutches were randomly selected for use in testing. It was not necessary to have males present during tests since all eggs produced throughout their reproductive life are fertilized during a single mating (Huizinga 1970).

All sediments were collected in January using a 0.1-m^2 van Veen bottom grab sampler. Contaminated test sediments (from the top 2-3 cm) were collected at a depth of 10 m from the Duwamish and Hylebos Waterways in Puget Sound, Washington. Both marine areas are heavily industrialized and have a history of contamination with a variety of aromatic hydrocarbons, chlorinated hydrocarbons and trace metals (Riley et al. 1981; Dexter et al. 1981; Malins et al. 1984). Moreover, previous studies have documented the toxic nature of these sediments (Swartz et al. 1982; Chapman and Morgan 1983; Schiewe et al. 1985). A relatively uncontaminated reference sediment was collected (top 2-3 cm) at a depth of 25 m from the mouth of the Dosewallips River Delta in Hood Canal, Washington, an area free of any known industrial inputs of chemical contaminants. To facilitate accurate counting of nauplii, tests were conducted using the finer component of the sediment; this allowed the nauplii (90 x 120 µm) to be separated from the sediment by screening. Sediments were rinsed with 3 µm filtered seawater through a series of three screens of 170, 93 and 64 µm mesh . The < 64 µm slurry was allowed to settle for 2 hr, overlying water was then siphoned away and aliquots of sediment were frozen at -20°C until tested. Chemical analysis was conducted on < 64 µm screened sediment using the methods described by MacLeod et al. (1985).

Test protocols utilized in this study are shown in Table 1. Every 7 d during testing females were removed from beakers with a pipette, and the beaker contents were

Table 1. Experimental protocols used to assess effect of contaminated sediment on naupliar production of *Tigriopus californicus*.

Experimental parameters	Experiment Number I	II	III	IV
Number females per beaker	20	1	5	5
Number of replicates per treatment				
Reference	1	13	6	5
Test	1	13	5	5
Beaker size (mL)	1000	150	150	150
Sediment source				
Reference	Dosewallips	Dosewallips	Dosewallips	Dosewallips
Test	Duwamish	Duwamish	Duwamish	Hylebos
Wet wt (g)[a] sediment per beaker	54	12	12	12
Total volume (mL) water, sediment and algal suspension per beaker	500	80	80	80
Test duration (wk)	10	9	3	3

[a] All sediment screened to <64 µm

rinsed through a 64 μm mesh screen, retaining all nauplii. Nauplii were rinsed into a petri dish, placed under a dissecting microscope, removed with a micropette and counted. Since nauplii of *T. californicus* are photonegative, a lamp directed at one side of the petri dish caused a mass movement to the opposite side of the dish, concentrating nauplii and facilitating counting. Seawater, algal suspension and freshly thawed sediment were then added to each beaker and allowed to settle for 1 hr; females were returned to their respective beakers. This procedure was repeated weekly. The mean number of nauplii per female each week was calculated and the mean total per female for the exposure period was statistically analyzed. At weekly intervals during exposure, the water column of three randomly selected beakers from control sediment and three from test sediment beakers were sampled for dissolved oxygen and pH. For all beakers dissolved oxygen remained between 4.4 to 6.6 mg/L, the median value was 5.8 mg/L; pH values ranged from 7.0 to 7.6, the median value was 7.2.

RESULTS AND DISCUSSION

Results of chemical analyses are shown in Table 2. Sediment from the Duwamish and Hylebos Waterways contained high levels of aromatic hydrocarbons, chlorinated hydrocarbons and metals. The Dosewallips Delta sediment was relatively uncontaminated, with one exception, elevated levels of manganese were detected.

Two long-term (9 and 10 wk) tests were conducted initially to define the pattern of naupliar production throughout the entire reproductive life span of *T. californicus*, and to determine if naupliar production was altered by exposure to contaminated sediments. This information was utilized to evaluate the feasibility of conducting shorter term tests.

Experiment I was conducted for 10 wk, by which time females had essentially ceased naupliar production. Results of weekly mean naupliar production by females exposed to sediment from the mouth of the Dosewallips River (reference) and Duwamish Waterway (test) are shown in Figure 1 (upper). Naupliar production by females on reference sediment was highest during the fourth wk with a single peak of 77 nauplii per female, and began to gradually decline thereafter. In contrast, reproduction of females on the contaminated Duwamish sediment was more irregular and peak naupliar production was delayed until wk 6 and 7, reaching a maximum of 55 nauplii per female. Over the 10 wk period each female on reference sediment produced a mean total of 337 nauplii. Females on contaminated sediment produced a mean total of 291 nauplii per female, a 13.6% reduction in offspring production over the entire reproductive life span.

Experiment II was conducted for 9 wk and was similar to the first experiment except individual females were isolated to improve statistical analysis. Naupliar production by females on Dosewallips sediment peaked at 70 nauplii per female during wk 2 and 3. In contrast, naupliar production of females on Duwamish sediment was, as in Experiment I, irregular with maximum naupliar production reaching 41 per female during wk 5 (Figure 1, lower). There was a significant difference in naupliar production between adult copepods on Dosewallips sediment and adult copepods on Duwamish sediment over time, (ANOVA, $p \leq 0.05$). A simple main-effects test (Keppel 1973) showed that the numbers of nauplii produced by females on Dosewallips sediment was significantly higher ($p \leq 0.05$) than females on Duwamish sediment during wk 1, 2, 3 and 4; there were no significant differences for wk 5, 6, 7, 8 or 9. Up to 4 wk, total naupliar production

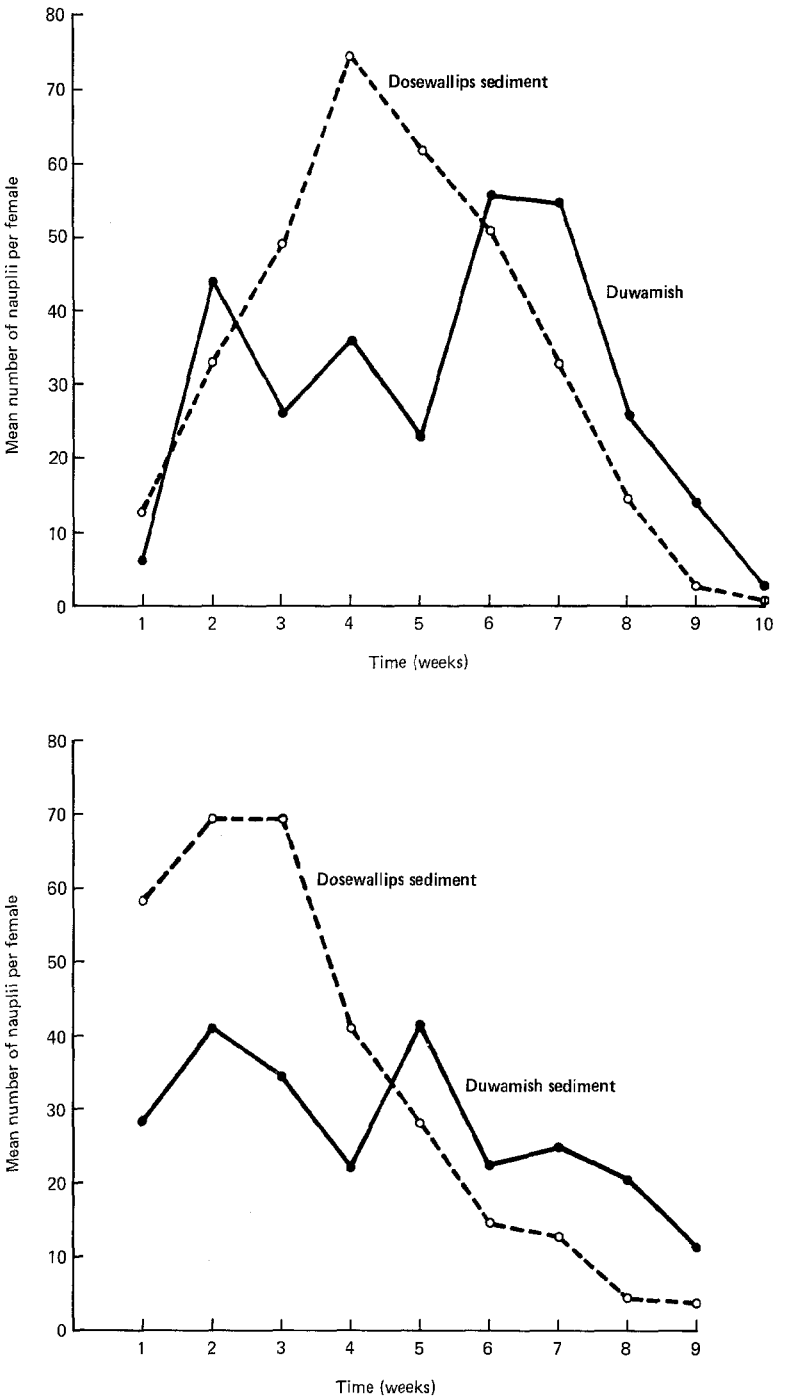

Figure 1. Mean number of nauplii produced by female *Tigriopus californicus* exposed to chemically contaminated Duwamish sediment and relatively uncontaminated Dosewallips sediment. (Upper): Experiment I. (Lower): Experiment II.

of females on Duwamish sediment was 46.8% lower than females held on Dosewallips sediment. Over the entire 9 wk, total naupliar production of females on Duwamish sediment was still significantly different ($p \leq 0.05$), but the difference had declined to 18%.

Table 2. Chemical characteristics of test and reference sediments. Analyses were conducted on a single homogeneous sample of three grabs from each site.

Compound(s)	Dosewallips Delta	Duwamish Waterway	Hylebos Waterway
Aromatic Hydrocarbons Σ (ng/g)[a] dry wt	Below Detection	17,000	24,000
Chlorinated Hydrocarbons Σ (ng/g)[b] dry wt	30	1,200	2,500
Metals Σ (µg/g) dry wt	1,500	3,400	3,600
Antimony	0.8	0.8	0.9
Arsenic	9	47	187
Cadmium	0.2	1	1.3
Chromium	114	172	155
Copper	62	825	599
Lead	10	164	158
Manganese	1180	1658	1634
Mercury	0.1	1.5	0.8
Nickel	44	45	302
Selenium	0.6	<0.1	0.8
Silver	<0.1	1	0.7
Tin	3	42	54
Zinc	73	411	460
Total Organic Carbon (%) wet wt[c]	0.9	0.5	4

[a] Summed concentration of 25 or more selected hydrocarbons.

[b] Summed concentration of 24 chlorinated hydrocarbons, including insecticides and polychlorinated biphenyls.

[c] Total organic carbon was determined on whole (unsieved) sediments.

The two long-term exposures encompassing the entire reproductive period showed similarities. Naupliar production in reference beakers peaked during the early weeks and then declined steadily until females reached 9-10 wk of age. Females exposed to test sediments produced nauplii in a more irregular pattern with overall lower peak production. The greatest differences in naupliar production between reference and test groups occurred during wk 3-4. Approximately 80% of the total naupliar production by gravid females exposed to reference sediments occurred during the first 4 wk; conversely, 80% of the total naupliar production among copepods exposed to test sediment occurred during the first 7 wk. Although the groups of copepods exposed to contaminated test sediment made a partial recovery in naupliar production during the last half of the reproductive period, the recovery was not sufficient to fully compensate for the larger reductions incurred during the early weeks. Ott et al. (1978) reported a similar response in the copepod

Eurytemora affinis exposed to naphthalene. Thus, results of the two long-term exposures demonstrated that sediment effects were most pronounced during the early weeks. This pattern suggested shorter-term (3 or 4 wk) tests could be used to demonstrate the toxic effects of contaminated sediments on reproduction.

In Experiment III we repeated the comparison of naupliar production on Dosewallips vs Duwamish sediments; however, the test duration was shortened to 3 wk. Females exposed to Dosewallips sediment produced a mean total of 117±19 nauplii per female, while females on Duwamish sediment produced a mean total of 48±12 nauplii per female, a reduction of 59%. ANOVA indicated these means were significantly different, ($p \leq 0.05$). In addition, the number of eggs per clutch was affected by exposure to contaminated sediment. After 3 wk, the number of eggs per clutch for females on Dosewallips sediment averaged 63 ± 10 (N=15) whereas, the number of eggs per clutch for females exposed to Duwamish sediment averaged 52 ± 7 (N=15). These means were significantly different, ($p \leq 0.05$). The lower number of eggs per clutch in Duwamish exposed females was a contributing factor to the observed reductions in naupliar production. Smaller brood sizes were also observed by Berdugo et al. (1977) when *E. affinis* was exposed to the water-soluble fraction of heating oil. A similar effect was reported by Ustach (1979) in the harpacticoid copepod *Nitocra affinis* exposed to the water-soluble fraction of Louisiana crude oil.

Experiment IV compared naupliar production of females on Dosewallips River (reference) and Hylebos Waterway (test) sediments for a 3 wk exposure. Females on Dosewallips sediment produced a mean total of 139 ± 17 nauplii, whereas females on Hylebos produced a mean total of 104 ± 22 nauplii. ANOVA showed that these means were significantly different, ($p \leq 0.05$) and represented a 25.2% reduction of nauplii for females on Hylebos Waterway sediment.

In summary, we found that exposure of ovigerous female *T. californicus* to two chemically contaminated marine sediments changed their pattern of naupliar production, delayed the time of peak production, and reduced the overall number of nauplii produced. These effects were observed in both 3 and 9 wk exposures. Based on these findings, this species appears to be a good candidate for use as a test organism in a chronic effects sediment bioassay.

Acknowledgments. We extend our thanks to several people for their most appreciated assistance: Eric Lynn of the NMFS, LaJolla, California for providing the initial stock culture of copepods and Jess Purdy of Southwest University for providing statistical analysis, Craig Haley and Sharon Demuth for providing technical assistance, and Doug Weber, Tracy Collier and Edmundo Casillas for reviewing the manuscript.

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Received August 8, 1989; accepted September 16, 1989.